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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Citron, et al.

Serial No.: 09/277,229   Group Art Unit No.: 1652

Filed: March 26, 1999   Examiner: Manjuanath N. Rao

For: Beta Secretase Genes and Polypeptides

Docket No.: A-581

DECLARATION UNDER 37 CFR 1.131

Commissioner for Patents
P.O. Box 1450
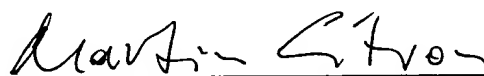Alexandria, VA 22313-1450
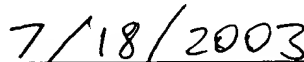
Sir:

I, Martin Citron, Ph.D., declare that:

1. I have been employed by Amgen Inc. since 1997 and currently hold the title of Associate Director II;

2. I am an applicant of the referenced patent application and a co-inventor of the claimed subject matter set forth therein;

3. I have reviewed the Official Action directed to the referenced patent application dated January 24, 2003,and I understand that the claims have been rejected under 35 USC 102(e) as lacking novelty in view of SEQ ID Nos: 4 and 8 disclosed in U.S. Patent Nos. 6,420,534 B1 and 6,440,698 B1. I understand that the '534 patent and the '698 patent both have a priority date of 24 September 1998.

4. I was the head of the beta-secretase project at Amgen at the time the invention as claimed in the referenced patent application was made;

5. I worked together with the other named inventors and their assistants to identify the beta secretase gene and polypeptide;

6. A true copy of the human beta secretase DNA sequencing data we obtained, together with a translation of such DNA in all 3 reading frames is attached hereto as Exhibit A and the true and correct sequence of beta secretase is set forth in the second reading frame, with the start and stop positions indicated.

7. Although the dates have been redacted in Exhibit A, the data set forth therein was all obtained prior to September 24, 1998.

I further declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both (18 USC 1001), and may jeopardize the validity of the application or any patent issuing thereon.

_____
Martin Citron, Ph.D.

7/18/2003
Date

Please send all future correspondence to:

US Patent Operations/NAO
Dept. 4300, M/S 27-4-A
AMGEN INC.
One Amgen Center Drive
Thousand Oaks, California 91320-1799

B.Bennett
DNA=HEK004008-C3
Assembled by CS

With 324 enzymes: `

MaxCuts: 4

```
                                                      EsiEI
                                                      EagI  |
              PbsI              Hin4I        SacII    |     |
              |                 |            |        |     |
       ACCTCCATAGAAGACACCCGGACCGATCCAGCCTCCCGCCCCGGGAACGGTGCCATTGGAA
       1 ---------+---------+---------+---------+---------+---------- 60
       TGGAGGTATCTTCTGTGGGCCTGGCTAGGTCGGAGGGCGGGGCCCTTGCCACGTAACCTT

    a      T  S  I  E  D  T  G  T  D  P  A  S  A  A  G  K  G  A  L  E  -
    b       P  P  *  K  T  P  G  P  I  Q  P  P  R  P  G  T  V  H  W  N -
    c        L  H  R  R  H  R  D  R  S  S  L  R  G  R  E  R  C  I  G  T-
```

```
              TfiI     BaeI                              PleI
          AloI |   BscGI  |                       BaeI   |
          | |  | |   |    |                       |      |
       CGCGGATTCCCCGTGCCAAGAGTGACGTAAGTACCGCCTATAGAGTCTATAGGCCCACCC
       61 ---------+---------+---------+---------+---------+---------+ 120
       GCGCCTAAGGGGCACGGTTCTCACTGCATTCATGGCGGATATCTCAGATATCCGGGTGGG

    a      R  G  F  P  V  P  R  V  T  *  V  P  P  I  E  S  I  G  P  P  -
    b       A  D  S  P  C  Q  E  *  R  K  Y  R  L  *  S  L  *  A  H  P -
    c        R  I  P  R  A  K  S  D  V  S  T  A  Y  R  V  Y  R  P  T  P-
```

```
                             MseI
         BstXI               VspI
         |                   |
       CCTTGGCTTCGTTAGAACGCCCCTACAATTAATACATAACCTTATGTATCATACACATAC
       121 ---------+---------+---------+---------+---------+---------- 180
       GGAACCGAAGCAATCTTGCGGGGATGTTAATTATGTATTGGAATACATAGTATGTGTATG

    a      P  W  L  R  *  N  A  A  T  I  N  T  *  P  Y  V  S  Y  T  Y  -
    b       L  G  F  V  R  T  R  L  Q  L  I  H  N  L  M  Y  H  T  H  T -
    c        L  A  S  L  E  R  G  Y  N  *  Y  I  T  L  C  I  I  H  I  R-
```

```
                                                  EsgI
                                                  |
       GATTTAGGTGACACTATAGAATAACATCCATTTGCCTTCTCTCCACAGGTGTCCACTC
       181 ---------+---------+---------+---------+---------+--------+ 240
       CTAAATCCACTGTGATATCTTATTGTAGGTAAACGGAAAGAGAGGTGTCCACAGGTGAG

    a      D  L  G  D  T  I  E  *  H  P  L  C  L  S  L  H  R  C  P  L  -
```

```
b    I * V T L * N   I H F A F L S T G V H S -
c    F R * H Y R I T S T L P F S P Q V S T P -
```

```
                                    HincII
                                    SimI
                                    AccI|
                      EcoRI   TaqI|   DrdI
                MneI    |     SalII|AflIII|
                NruI |  |     HgaI||| MluI|  AceIII
                  | |  |      |||!     ||      |
        CCAGGTCCAACTGCACCTCGGTTCTATCGCGATTGAATTCGTCGACCCAGCGTCCGCAG
   241  ---------+---------+---------+---------+---------+---------+  300
        GGTCCAGGTTGACGTGGAGCCAAGATAGCGCTAACTTAAGCAGCTGGGTCGCAGGCGTC
```

```
a    P G P T A P R F Y R D * I R R P T R P Q -
b    Q V Q L H L G S I A I E F V D P R V R S -
c    R S N C T S V L S R L N S S T H A S A A -
```

```
                   AceIII
            SmaI     |
            AvaI |   |
              | |    |                     Bpu10I
              | |    |                        |
        CCCGCCCGGGAGCTGCGAGCCCGGACCTGGATTATGGTGGCCTGAGCAGCCAACGCAGCC
   301  ---------+---------+---------+---------+---------+---------+  360
        GGGCGGGCCCTCGACGCTCGGGCCTGGACCTAATACCACCGGACTCGTCGGTTGCGTCGG
```

```
a    P A R E L R A A S W I M V A * A A N A A -
b    P P G S C E P R A G L W W P E Q P T Q P -
c    R P G A A S R E L D Y G G L S S Q R S R -
```

```
        GCAGGAGCCCGGAGCCCTTGCCCCTGCCCGCGCCGCGCCGGCGCGGGCGGACCAGGGAAG
   361  ---------+---------+---------+---------+---------+---------+  420
        CGTCCTCGGGCCTCGGGAACGGGGACGGGCGCGGCGCGGCCGCGCCCGCCTGGTCCCTTC
```

```
a    A G A R S P C P C P R R R P P G G P G K -
b    Q E P G A L A P A R A A A R R G D Q G S -
c    R S P E P L P L P A P P P A G G T R E A -
```

```
        BsrFI
          |
        CCGGCACCGGCCGGCCATGCCCGGCCCTCCCAGCCCGGCCGGCGAGCCCGCGCCCGCTGCC
   421  ---------+---------+---------+---------+---------+---------+  480
        GGCCGTGGCCGGCCGGTACGGGCCGGGAGGGTCGGGCCGGCCGCTCGGGCGCGGGCGACGG
```

```
a    P P P A R H A R P S Q P R R E P A P A A -
b    R H R P A M P A P P S P A G S P R P L P -
c    A T G P P C P P L P A P P G A R A R C P -
```

```
                SamHI
                BstYI
              BsaWI |
              BspEI |                       BscGI
                |   |                          |
        CAGGCCTGGCCGGCCGGTGCCGATGTAGCGGGCTCCGGATCCCAGCCCTCTCCCCTGCTCC
   481  ---------+---------+---------+---------+---------+---------+  540
```

```
        GTCCGACCGGCGGCGGCAGCGGCTACATCGCCCCGAGGCCTACGGTCGGAGAGGGGACGAGG
```

```
a     Q  A  G  R  R  R  A  D  V  A  G  S  G  S  Q  P  L  P  C  S  -
b     R  L  A  A  A  V  P  M  *  R  A  P  D  P  S  L  S  P  A  P  -
c     G  W  P  P  P  C  R  C  S  G  L  R  I  P  A  S  P  L  L  P  -
```

```
                                                          SraI
                                                    AvaI  |
              BstYI                          SmlI    |    |
    BsiHKAI    '              BsrBI           |       ||
       |       :                |             |       |||
    CGTGCTCTGCGGATCTCCCTGACCGGCTCTCCACAGCCCGGACCCGGGGGCTGCCCCAGG
541 ---------+---------+---------+---------+---------+--------- 600
    GCACCGAGACGCCTAGAGGGACTGGCCGAGAGGTGTCGGGCCTGGGCCCCCGACGGGGTCC
```

```
a     R  A  L  R  I  S  P  D  R  S  P  Q  P  G  P  G  G  W  P  R  -
b     V  L  C  G  S  P  L  T  A  L  H  S  P  D  P  G  A  G  P  G  -
c      C  S  A  D  L  P  *  P  L  S  T  A  R  T  R  G  L  A  Q  G  -
```

```
              PstI
              SbfI
              SfiI |
          HgaI  | |
       ApaI |   | |        BsaHI
         |  |   | |          |
    GCCCTGCACGCCCTGGCGTCCTGATGCCCCCCAAGCTCCCTCTCCTGAGAAGCCACCAGCA
601 ---------+---------+---------+---------+---------+---------+ 660
    CGGGACGTGCGGGACCGCAGGACTACGGGGGGTTCGAGGGAGAGGACTCTTCGGTGGTCGT
```

```
a     A  L  Q  A  L  A  S  *  C  P  Q  A  P  S  P  E  K  P  P  A  -
b     P  C  R  P  W  R  P  D  A  P  K  L  P  L  L  R  S  H  Q  H  -
c     P  A  G  P  G  V  L  M  P  P  S  S  L  S  *  E  A  T  S  T  -
```

```
                        HaeII
                  BsaHI   |
                  NarI    |
                   |      |
    CCACCCAGACTTGGGGGCAGGATGCCAGGGACGGACGTGGGCCAGTGCGAGCCCAGAGGGC
661 ---------+---------+---------+---------+---------+---------+ 720
    GGTGGGTCTGAACCCCCGTCCTACGGTCCCTGCCTGCACCCGGTCACGCTCGGGTCTCCCG
```

```
a     P  P  R  L  G  G  R  R  Q  G  R  T  W  A  S  A  S  P  E  G  -
b     H  P  D  L  G  A  G  A  R  D  G  R  G  P  V  R  A  Q  R  A  -
c      T  Q  T  W  G  Q  A  P  G  T  D  V  G  Q  C  E  P  R  G  P  -
```

```
                        NcoI
    ApaI         ApaI    |
      |            |     |
    CCGAACGGCCGGGGCCCACCATGGCCCAAGCCCTGCCCTGGCTCCTGCTGTGGATGGGCGC
721 ---------+---------+---------+---------+---------+---------+ 780
    GGCTTGCCGGCCCCCGGGTGGTACCGGGTTCGGGACGGGACCGAGGACGACACCTACCCGCG
```

```
a     P  K  A  G  A  H  H  G  P  S  P  A  L  A  P  A  V  D  G  R  -
b     R  R  P  G  P  T  Ⓜ  A  Q  A  L  P  W  L  L  L  W  M  G  A  1
c     E  G  R  G  P  P  W  P  K  P  C  P  G  S  C  C  G  W  A  R  -
```

                    ↓ Start

Fsp1

```
            GGGAATTGCTTGCCTGCAGGCGTANTAGCACGGCATCCTGCTGCCCTTGCGGAGCGGCCT
        781 .....................................................------- 940
            CCCTTAACGAACGGACGGTCCGCATANTCGTGCCGTAGGACGACGGGAACGCCTCGCCGGA
```

a   C S A A C F F H F A H H P A A F A L R P -
b   G V L P A H G T Q H G I R L P L R S G L -G
c   E C C L P T A P S T A S P C F C A A A W -

```
        HaeII                    Brel
    BsaHI    |              Aval        EaeI
    NarI     |              BsaI|       SapI          Aval
    |    |                       |   |                 |
            GCCGGTTCTTCCCTTGGGTGCCAATGCCGGGAAGCTTGTAGCAGTGCGTGACGAGCGGC
        941 --...----------------+----------+-------------+---------- 960
            CGCCCAAGAAGGGAACCCACGGTTACGGCCCTTCGAACATCGTCACGCACTGCTCGCCG
```

a   G G R P P G A A A A P G D R R R A R G A -
b   G G A P L G L R L P R E T D E E P E E P -
c   G A P P W G C G C P G R P T K S P R S P -

```
        BseRI
    BsiEI   |
    EagI  |   |                    Bsu36I
          |   |                       |
            CGGCCGTGAAGGGCAGTTTGTGGAGATGGTGGACTAACTGAGGGGCAAGTCGGGCCAGGG
        901 ----------+---------+----------------------------+-------- 960
            GCCGGCACTTCCCGTCAAACACCTCTACCACCTGATTGACTCCCCGTTCAGCCCGGTCCC
```

a   R P E G Q L C G D G G Q P E G Q V G A G -
b   G R R G S F V E M V D N L R G K S G Q G -G
c   A G G A A L W R W W T T * G A S R G R A -

```
                                            MslI
                                            BciVI |
                                    HgaI      |  ||
            BsaAI                     |        |  ||
            |                                  |  ||
            CTACTACGTGGAGATGACCGTGGGCATCCCTCTGCAGAGCGTCAACATCCTGGTGGAGATAC
        961 -------------------+----------+---------+------------------ 1020
            GATGATGCACCTCTACTGGCACCCGTAGGGAGACGTCTCGCAGTTGTAGGACCACCTCTATG
```

a   L L R G D D R G Q P P A D A Q H P G G Y -
b   Y Y V E M T V G S P P Q T L N I L V E M T
c   T T W R * P W A A P F R R S T S W W I Q -

```
            EstAPI
    BtsI     |
    |    |
            AGGCAGTAGTTAACTTTCCAGTGGGTGCTGCCCCCACCCGGTTGCTGCATCGCTACTACCA
        1021 ------------------------+---------+----------+----------+ 1080
            TCCGTCGTCATTGAAAGGTCACCCACGACGGGGGTGGGCCAACGACGTAGCGATGATGGT
```

a   R Q Q * L C S G C C P P P L P A S L L P -
b   G S V S I N F A V G A A P H P F L H R Y Y Q -G

```
c     A  A  V  T  L  Q  W  V  L  P  P  T  P  S  C  I  A  T  T  R  -

                                          EcoNI
                                   BsaWI
                   BsoGI           BspEI  |
             PvuII   |       Psp5II |     |
               |     |         |    |     |
     GAGGCAGCTGTCCAGCACATACCGGGACCTCCGGAAGGGTGTGTATGTGCCCTACACCCA
1081 --------+---------+---------+---------+---------+---------+ 1140
     CTCCGTCGACAGGTCGTGTATGGCCCTGGAGGCCTTCCCACACATACACGGGATGTGGGT

a     E  A  A  V  Q  H  I  P  G  P  P  E  G  C  V  C  A  L  H  P  -
b     R  Q  L  S  S  T  Y  R  D  L  R  K  G  V  Y  V  P  Y  T  Q  -
c     G  S  C  P  A  H  T  G  T  S  G  R  V  C  M  C  P  T  P  R  -

                                 SexAI          NcoI
         AceIII                    |             |
           |                       |             |
     GGGCCAAGTGGGAAGGGGAGCTGGGCACCGACCTGGTAAGCATCCCCCATGGCCCCAACGT
1141 --------+---------+---------+---------+---------+---------+ 1200
     CCCGGTTCACCCTTCCCCCTCGACCCGTGGCTGGACCATTCGTAGGGGGTACCGGGGTTGCA

a     G  Q  V  G  R  G  A  G  H  R · P  G  K  H  P  P  W  P  Q  R  -
b     G  K  W  E  G  E  L  G  T  D  L  V  S  I  P  H  G  P  N  V  -
c     A  S  G  K  G  S  W  A  P  T  W  *  A  S  P  M  A  P  T  S  -

                                      TfiI
         EcoK12  BsrDI                 |
           |       |                   |
     CACTGTGCGTGCCAACATTGCTGCCATCACTGAATCAGACAAGTTCTTCATCAACGGCTC
1201 --------+---------+---------+---------+---------+---------+ 1260
     GTGACACGCACGGTTGTAACGACGGTAGTGACTTAGTCTGTTCAAGAAGTAGTTGCCGAG

a     H  C  A  C  Q  H  C  C  H  H  *  I  R  Q  V  L  H  Q  R  L  -
b     T  V  R  A  N  I  A  A  I  T  E  S  D  K  F  F  I  N  G  S  -
c     L  C  V  P  T  L  L  P  S  L  N  Q  T  S  S  S  T  A  P  -

                                             PleI
                                           (StuI)  ?
         BfiI          MmeI                  |
           |            |                    |
     CAACTGGGAAGGCATCCTGGGGCTGGCCTATGCTGAGATTGCCAGGCCTGACGACTCCCT
1261 --------+---------+---------+---------+---------+---------+ 1320
     GTTGACCCTTCCGTAGGACCCCGACCGGATACGACTCTAACGGTCCGGACTGCTGAGGGA

a     Q  L  G  R  H  P  G  A  G  L  C  *  D  C  Q  A  *  R  L  P  -
b     N  W  E  G  I  L  G  L  A  Y  A  E  I  A  R  P  D  D  S  L  -
c     T  G  K  A  S  W  G  W  P  M  L  R  L  P  G  L  T  T  P  W  -

                                              EarI
         PleI           BgmI     SimI      BsaXI  |    PstI
           |              |        |          |   |      |
     GGAGCCTTTCTTTGACTCTCTGGTAAAGCAGACCCACGGTTCCCAACCTCTTCTCCCTGCA
1321 --------+---------+---------+---------+---------+---------+ 1380
     CCTCGGAAAGAAACTGAGAGACCATTTCGTCTGGGTGCAAGGGTTGGAGAAGAGGGACGT

a     G  A  F  L  *  L  S  G  K  A  D  P  R  S  Q  P  L  L  P  A  -
b     E  P  F  F  D  S  L  V  K  Q  T  H  V  P  N  L  F  S  L  Q  -
```

```
         c      S  L  S  L  T  L  W  *  S  R  P  T  F  P  T  S  S  P  C  S  -

                                 MneI                    Eco57I
                                  |                        :
              GCTTTGTGGTGCTGGCTTCCTCCTCAACCAGTCTGAAGTGCTGGCCTCTGTCGGAGGGAG
       1381  ---------+---------+---------+---------+---------+---------+  1440
              CGAAACACCACGACCGAAGGAGGAGTTGGTCAGACTTCACGACCGGAGACAGCCTCCCTC

         a      A  L  W  C  W  L  P  P  Q  P  V  *  S  A  G  L  C  R  R  E    -
         b      L  C  G  A  G  F  P  L  N  Q  S  E  V  L  A  S  V  G  G  S    -
         c      F  V  V  L  A  S  P  S  T  S  L  K  C  W  P  L  S  E  G  A    -

                                         ┌──────┐
                                         │ BsrGI│              BstZ17I
               BclI            TaqI       │ TatI │               AccI
                .                |        └──┼───┘                | :
              CATGATCATTGGAGGTATCGACCACTCGCTGTACACAGGCAGTCTCTGGTATACACCTAT
       1441  ---------+---------+---------+---------+---------+---------+  1500
              GTACTAGTAACCTCCATAGCTGGTGAGCGACATGTGTCCGTCAGAGACCATATGTGGATA

         a      H  D  H  W  R  Y  R  P  L  A  V  H  R  Q  S  L  V  Y  T  H    -
         b      M  I  I  G  G  I  D  H  S  L  Y  T  G  S  L  W  Y  T  P  I    -'
         c      *  S  L  E  V  S  T  T  R  C  T  Q  A  V  S  G  I  H  P  S    -

                                 BclI                         BstYI
                                  |                            |
              CCGGCCGGGAGTGGTATTATGAGGTGATCATTGTGCGGGTGGAGATCAATGGACAGGATCT
       1501  ---------+---------+---------+---------+---------+---------+  1560
              GGCCGGCCCTCACCATAATACTCCACTAGTAACACGCCCACCTCTAGTTACCTGTCCTAGA

         a      P  A  G  V  V  L  *  G  D  H  C  A  G  G  D  Q  W  T  G  S    -
         b      R  R  E  W  Y  Y  E  V  I  I  V  R  V  E  I  N  G  Q  D  L   -Q
         c      G  G  S  G  I  M  R  *  S  L  C  G  W  R  S  M  D  R  I  *    -

                          TatI
                           |
              GAAAATGGACTGCAAGGAGTACAACTATGACAAGAGCATTGTGGACAGTGGCACCACCAA
       1561  ---------+---------+---------+---------+---------+---------+  1620
              CTTTTACCTGACGTTCCTCATGTTGATACTGTTCTCGTAACACCTGTCACCGTGGTGGTT

         a      E  N  G  L  Q  G  V  Q  L  *  Q  E  H  C  G  Q  W  H  H  Q    -
         b      K  M  D  C  K  E  Y  N  Y  D  K  S  I  V  D  S  G  T  T  N   -7
         c      K  W  T  A  R  S  T  T  M  T  R  A  L  W  T  V  A  P  P  T    -

               Tth111II                    PstI        BseRI
                  |                          |           |
              CCTTCGTTTGCCCAAGAAAGTGTTTGAAGCTGCAGTCAAATCCATCAAGGCAGCCTCCTC
       1621  ---------+---------+---------+---------+---------+---------+  1680
              GGAAGCAAACGGGTTCTTTCACAAACTTCGACGTCAGTTTAGGTAGTTCCGTCGGAGGAG

         a      P  S  F  A  Q  E  S  V  *  S  C  S  Q  I  H  Q  G  S  L  L    -
         b      L  R  L  P  K  K  V  F  E  A  A  V  K  S  I  K  A  A  S  S   -8
         c      F  V  C  P  R  K  C  L  K  L  Q  S  N  P  S  R  Q  P  P  P    -

                                 BfaI        PvuII
                                  |           |
```

```
        CAGGGAGAAGTTCTCTGATGGTTTCTGGCTAGGAGAGCAGGTGGTGTGTGGCAAGCACG
  1581  -------------------------+---------+--------+--------------+  1740
        GTCCCTCTTCAAGGGACTACCAAAGACCGATCCTCTCGTCGACCACACGCCGTTCGTCC

a     H  G  E  V  P  *  W  F  L  A  R  R  A  A  G  V  L  A  S  R  -
b     T  E  K  F  P  D  G  F  W  L  G  E  Q  L  V  @  W  Q  A  G  -
c     R  R  S  S  L  M  V  S  G  *  E  S  S  W  C  A  G  R  Q  A  -


            PflMI        XmnI
          Tth111I      PflI
                                                              BstEII
                           |
        CAGCACCCCCTTGGAACATTTTCCCCATTCATCTGCTCTCGTTAATGGGTGACCGTTACCCA
  1741  --------+---------+---------+---------+------+---------+----+  1800
        GTCGTGGGGGAACCTTGTAAAAGGGGTCAGTAGACGAGAGCAATTACCCACTGGCAATGGGT

a     H  H  P  L  E  H  F  P  H  L  T  L  P  N  G  *  G  Y  Q
b     T  T  P  W  N  I  F  P  V  I  S  L  Y  L  M  G  E  V  T  N  -
c     P  P  L  G  T  F  S  Q  S  S  H  S  T  *  W  V  R  I  P  T


                              BstAPI    BspMI            MscI
                                                           |
        CCAGTCCTTCCGGCATCACCATCCTTCCGGCAGCAATACCTGCGGGCCAGTGGAAGATGTGGC
  1801  ---------+---------+---------+---------+---------+---------+  1860
        GGTCAGGAAGGCCGTAGTGGTAGGAAGGCCGTCGTTATGGACGCCCGGTCACCTTCTACACCG

a     P  V  L  P  H  H  H  P  S  A  A  I  P  A  A  S  G  R  C  G  -
b     Q  S  F  R  I  T  I  L  P  Q  Q  Y  L  R  P  V  E  D  V  A  -
c     S  P  S  A  S  P  S  F  R  S  N  T  C  G  Q  W  K  M  W  P  -


                                          AceIII
                                        BscGI | MslI
                                          :   |    |
        CACGTCCCAAGACGACTGTTACAAGTTTGCCATCTCACAGTCATCCACGGGCACTGTTAT
  1861  ---------+---------+---------+---------+---------+---------+  1920
        GTGCAGGGTTCTGCTGACAATGTTCAAACGGTAGAGTGTCAGTAGGTGCCCGTGACAATA

a     H  V  P  R  R  L  L  Q  V  C  H  L  T  V  I  H  G  H  C  Y  -
b     T  S  Q  D  D  @  Y  K  F  A  I  S  Q  S  S  T  G  T  V  M  -?
c     R  P  K  T  T  V  T  S  L  P  S  H  S  H  P  R  A  L  L  W  -


          Hin4I                          ApaI
            |                             |
        GGGAGCTGTTATCATGGAGGGCTTCTACGTTGTCTTTGATCGGCGCCCGAAAACGAATTGG
  1921  ---------+---------+---------+---------+---------+---------+  1980
        CCCTCGACAATAGTACCTCCCGAAGATGCAACAGAAACTAGCCGCGGGCTTTTGCTTAACC

a     G  S  C  Y  H  G  G  L  L  R  C  L  *  S  G  P  K  T  N  W  -
b     G  A  V  I  M  E  G  F  Y  V  V  F  D  R  A  R  K  R  I  G  -?
c     E  L  L  S  W  R  A  S  T  L  S  L  I  G  P  E  N  E  L  A  -


                    HaeII    BsiHKAI
          Eco47III    |     ApaLI  |
                      |        |   |
        CTTTCCTGTCAGCGCTTGCACATGTGCACGATGAGTTCAGGACGGCAGCGGCTGGCAGGCCC
  1981  ---------+---------+---------+---------+---------+---------+  2040
```

GAAACGACAGTGCGGAACGGTACAGGTGCTACTCAAGTCCTGCCGGTCGCCATTTCCAGG

```
a    L  C  C  Q  R  L  P  C  A  R  *  V  Q  D  G  S  G  G  R  P  -
b    F  A  V  S  A  C  E  V  H  D  E  F  R  T  A  A  V  E  G  P  -
c    L  L  S  A  L  A  M  C  T  M  S  S  G  R  Q  R  W  K  A  L  -
```

                                                    HincII
                        EbsI   DrdI |

```
     TTTTGTTCACCTTGGACATGGAAGACTGTGGCTACAACATTCCACAGACACATGAGTCAAC   2100
2041 --------------------------------------+--------------------
     AAAACAGTGGAACCTGTACCTTCTGACACCGATGTTGTAAGGTGTCTGTGTACTCAGTTG
```

```
a    F  C  H  L  G  H  G  R  L  W  L  Q  H  S  T  D  E  *  V  N  -
b    F  V  T  L  D  M  E  D  C  G  Y  N  I  P  Q  T  D  E  S  T  -
c    L  S  P  W  T  W  K  T  V  A  T  T  F  H  R  Q  M  S  Q  P  -
```

     RcaI   EarI
     PleI | |

```
     CCTCATGACCATAGCCTATGTCATGGCTGCCATCTGCGCCCTCTTCATGCTGCCACTCTG   2160
2101 ------------------------+------------------------+---------+
     GGAGTACTGGTATCGGATACAGTACCGACGGTAGACGCGGGAGAAGTACGACGGTGAGAC
```

```
a    P  H  D  H  S  L  C  H  G  C  H  L  R  P  L  H  A  A  T  L  -
b    L  M  T  I  A  Y  V  M  A  A  I  C  A  L  F  M  L  P  L  Q  -?
c    S  *  P  *  P  M  S  W  L  P  S  A  P  S  S  C  C  H  S  A  -
```

                      HaeII
                       |

```
     CCTCATGGTGTGTCAGTGGCGCTGCCTCCGCTGCCTGCGGCCAGCAGCATGATGACTTTGC   2220
2161 --------------------+----------------+---------------------+
     GGAGTACCACACAGTCACCGCGACGGAGGCGACGGACGCCGGTCGTCGTACTACTGAAACG
```

```
a    P  H  G  V  S  V  A  L  P  P  L  P  A  P  A  A  *  *  L  C  -
b    L  M  V  Q  Q  W  R  C  L  R  Q  L  R  Q  Q  H  D  D  F  A  -  ?
c    S  W  C  V  S  G  A  A  S  A  A  C  A  S  S  M  M  T  L  L  -
```

                                BseRI
                                Eco57I
                        NcoI || TfiI   BspGI
     Hin4I | || | |

```
     TGATGACATCTCCCTGCTGAAGTGAGGAGGCCCATGGGCAGAAGATAGAGATTCCCCTGG   2280
2221 ----------+------------------+----------+--------------------
     ACTACTGTAGAGGGACGACTTCACTCCTCCGGGTACCCGTCTTCTATCTCTAAGGGGACC
```

```
a    *  *  H  L  P  A  E  V  R  R  P  M  G  R  R  *  R  F  P  W  -
b    D  D  I  S  L  L  K  *  G  G  P  W  A  E  D  R  D  S  P  G  -
c    M  T  S  P  C  *  S  E  E  A  H  G  Q  K  I  E  I  P  L  D  -
```

     BsaXI   MscI
     Hin4I MslI DrdII |

                              Stop

```
     AGCACACCTCCGTGGTTCACTTTGGTCACAAGTAGGAGACACAGATGGCACCTGTGGCCA   2340
2281 ----------------------+----------+--------------------------+
```

Wilne

```
                TGGTGTGGAGGCACCAAGTGAAACCAGTGTTCATCCTCTGTGTGTCTACCGTGGACACCGGT

   a       T  T  P  P  W  F  T  L  V  T  S  R  R  H  R  W  H  L  W  P   -
   b       P  H  L  R  G  S  L  W  S  Q  V  G  D  T  D  G  T  C  G  Q   -
   c       H  T  S  V  V  H  F  G  H  K  *  E  T  Q  M  A  P  V  A  R   -


                      Psp5II
            Bsu36I       |
         BsiHKAI    |SimI
              |     |    |
            GAGCACCTCAGGACCCTCCCCACCCACCAAATGCCTCTGCCTTGATGGAGAAGGAAAGG
   2341     --------+---------+---------+---------+---------+---------+  2400
            CTCGTGGAGTCCTGGGAGGGGTGGGTGGTTTACGGAGACGGAACTACCTCTTCCTTTTCC

   a       E  H  L  R  T  L  P  T  H  Q  M  P  L  P  *  W  R  R  K  R   -
   b       S  T  S  G  P  S  P  P  T  K  C  L  C  L  D  G  E  G  K  G   -
   c       A  P  Q  D  P  P  H  P  P  N  A  S  A  L  M  E  K  E  K  A   -


            CTGGCAAGGTGGGTTCCAGGGACTGTACCTGTAGGAAACAGAAAAGAGAAGAAAGAAGCA
   2401     --------+---------+---------+---------+---------+---------+  2460
            GACCGTTCCACCCAAGGTCCCTGACATGGACATCCTTTGTCTTTTCTCTTCTTTCTTCGT

   a       L  A  R  W  V  P  G  T  V  P  V  G  N  R  K  E  K  K  E  A   -
   b       W  Q  G  G  F  Q  G  L  Y  L  *  E  T  E  K  R  R  K  K  H   -
   c       G  K  V  G  S  R  D  C  T  C  R  K  Q  K  R  E  E  R  S  T   -


                                                    Eco57I
                  BstEII           MseI Tth111II       |
                    |                |    |            |
            CTCTGCTGGCGGGAATACTCTTGGTCACCTCAAATTTAAGTCGGGAAATTCTGCTGCTTG
   2461     --------+---------+---------+---------+---------+---------+  2520
            GAGACGACCGCCCTTATGAGAACCAGTGGAGTTTAAATTCAGCCCTTTAAGACGACGAAC

   a       L  C  W  R  E  Y  S  W  S  P  Q  I  *  V  G  K  F  C  C  L   -
   b       S  A  G  G  N  T  L  G  H  L  K  F  K  S  G  N  S  A  A  *   -
   c       L  L  A  G  I  L  L  V  T  S  N  L  S  R  E  I  L  L  L  E   -


                                         DraI
                                         MseI|
                                          ||
            AAACTTCAGCCCTGAACCTTTGTCCACCATTCCTTTAAATTCTCCAACCCAAAGTATTCT
   2521     --------+---------+---------+---------+---------+---------+  2580
            TTTGAAGTCGGGACTTGGAAACAGGTGGTAAGGAAATTTAAGAGGTTGGGTTTCATAAGA

   a       K  L  Q  P  *  T  F  V  H  H  S  F  K  F  S  N  P  K  Y  S   -
   b       N  F  S  P  E  P  L  S  T  I  P  L  N  S  P  T  Q  S  I  L   -
   c       T  S  A  L  N  L  C  P  P  F  L  *  I  L  Q  P  K  V  F  F   -


                        ScaI
              MseI      TatI |BspMI        BstEII
                |         |  | |      |       |
            TCTTTTCTTAGTTTCAGAAGTACTGGCATCACACGCAGGTTACCTTGGCGTGTGTCCCTG
   2581     --------+---------+---------+---------+---------+---------+  2640
            AGAAAAGAATCAAAGTCTTCATGACCGTAGTGTGCGTCCAATGGAACCGCACACAGGGAC
```

```
a    S F L S F R S T G I T R R L P W R V S L -
b    L F L V S E V L A S H A G Y L G V C P C -
c    F S * F Q K Y W H H T Q V T L A C V P V -

              BsaI
         KpnI EarI |        HindIII              MscI
          |    |   |           |                  |
         TGGTACCCTGGCAGAGAAGAGACCAAGCTTGTTTCCCTGCTGGCCAAAGTCAGTAGGAGA
    2641 --------+---------+---------+---------+---------+---------+ 2700
         ACCATGGGACCGTCTCTTCTCTGGTTCGAACAAAGGGACGACCGGTTTCAGTCATCCTCT

a    W Y P G R E E T K L V S L L A K V S R R -
b    G T L A E K R P S L F P C W P K S V G E -
c    V P W Q R R D Q A C F P A G Q S Q * E R -

             BstAPI
               |
         GGATGCACAGTTTGCTATTTGCTTTAGAGACAGGGACTGTATAAACAAGCCTAACATTGG
    2701 --------+---------+---------+---------+---------+---------+ 2760
         CCTACGTGTCAAACGATAAACGAAATCTCTGTCCCTGACATATTTGTTCGGATTGTAACC

a    G C T V C Y L L * R Q G L Y K Q A * H W -
b    D A Q F A I C F R D R D C I N K P N I G -
c    M H S L L F A L E T G T V * T S L T L V -

                                                    BsiEI
                                              EagI   |
                                              NotI?  |
         BstAPI          MseI          BfaI     |    |
           |              |             |       |    |
         TGCAAAGATTGCCTCTTGAATTAAAAAAAAAAACTAGAAAAAAAAAAAAAAAGGGCGGCC
    2761 --------+---------+---------+---------+---------+---------+ 2820
         ACGTTTCTAACGGAGAACTTAATTTTTTTTTTTGATCTTTTTTTTTTTTTTTCCCGCCGG

a    C K D C L L N * K K K L E K K K K R A A -
b    A K I A S * I K K K N * K K K K K G R P -
c    Q R L P L E L K K K T R K K K K K G G R -

               HindIII
          EcoRV   |
          Taq I | |        Sfi I
           |   | |     NcoI |
          GCAAATTCGATATCAAGCTTGGCCGGCCATGGCCCAACTTGTTTATTGCAGCTTATAATGG
    2821 --------+---------+---------+---------+---------+---------+ 2880
          CGTTTAAGCTATAGTTCGAACCGGCCGGTACCGGGTTGAACAAATAACGTCGAATATTACC

a    A N S I S S L A A M A Q L V Y C S L * W -
b    Q I R Y Q A W P P W P N L F I A A Y N G -
c    K F D I K L G R H G P T C L L Q L I M V -

                                                  BsmI
                                             BtsI |  BfaI
                                               |  |   |
         TTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTC
    2881 --------+---------+---------+---------+---------+---------+ 2940
         AATGTTTATTTCGTTATCGTAGTGTTTAAAGTGTTTATTTCGTAAAAAAAGTGACGTAAG
```

```
a      L  Q  I  K  Q  *  H  H  K  F  H  K  *  S  I  F  F  T  A  F  -
b      Y  K  *  S  N  S  I  T  N  F  T  N  K  A  F  F  S  L  H  S  -
c      T  N  K  A  I  A  S  Q  I  S  Q  I  K  H  F  F  H  C  I  L  -

          TAGTTGTGGTTTGTCCAAACTCATC
    2941  ---------+---------+----- 2965
          ATCAACACCAAACAGGTTTGAGTAG

a      *  L  W  F  V  Q  T  H    -
b      S  C  G  L  S  K  L  I    -
c      V  V  V  C  P  N  S    -
```

Enzymes that do cut and were not excluded:

EcoK12	AccI	AceIII	AflIII	AloI	ApaI	ApaLI	AvaI
BaeI	BamHI	BbsI	BciVI	BclI	BfaI	BfiI	BgmI
Bpu10I	BsaI	BsaAI	BsaHI	BsaWI	BsaXI	BscGI	BseRI
BsgI	BsiEI	BsiHKAI	BsmI	BspEI	BspGI	BspMI	BsrBI
BsrDI	BsrFI	BsrGI	BstAPI	BstEII	BstXI	BstYI	Bst217I
Bsu36I	BtsI	DraI	DrdI	DrdII	EagI	EarI	Eco47III
Eco57I	EcoNI	EcoRI	EcoRV	FspI	HaeII	HgaI	Hin4I
HincII	HindIII	KpnI	MluI	MmeI	MscI	MseI	MslI
NarI	NcoI	NotI	NruI	PflMI	PleI	Psp5II	PstI
PvuII	RcaI	SacII	SalI	SapI	SbfI	ScaI	SexAI
SfiI	SimI	SmaI	StuI	TaqI	TatI	TfiI	Tth111II
VspI	XmnI						

Enzymes that do not cut:

AarI	AatII	AclI	AflII	AhdI	AscI	AvrII	BbvCI
Bce83I	BcgI	BglII	BplI	Bpu1102I	BsaBI	BsbI	BsmBI
Bsp24I	BspLU11I	BssHII	BssSI	ClaI	DraIII	EciI	FseI
HaeIV	HgiEII	HpaI	MfeI	NdeI	NgoAIV	NheI	NsiI
NspI	NspV	PacI	Pfl1108I	PinAI	PmeI	PmlI	PshAI
PvuI	RleAI	RsrII	SacI	SanDI	SgfI	SgrAI	SmlI
SnaBI	SpeI	SphI	SrfI	Sse8647I	SspI	StuI	SwaI
Tth111I	UbaLI	XbaI	XcmI	XhoI			

Enzymes excluded; MinCuts: 1 MaxCuts: 4

AciI	AluI	AlwI	AlwNI	ApoI	AvaII	BanI	BanII
BbvI	BccI	BcefI	BglI	BmgI	BsaJI	BseMII	BseSI
BslI	BsmAI	BsmFI	Bsp1286I	BsrI	Bst4CI	BstDSI	Cac8I
CjeI	CjeI	CjePI	CjePI	CviJI	CviRI	DdeI	DpnI
EaeI	Eco0109I	EcoRII	FauI	Fnu4HI	FokI	GdiII	HaeI
HaeIII	HhaI	HinfI	HphI	MaeIII	MboII	MnlI	MspI
MspA1I	MwoI	NciI	NlaIII	NlaIV	RsaI	Sau96I	Sau3AI
ScrFI	SfaNI	SfcI	StyI	TaiI	TaqII	TaqII	TauI
ThaI	TseI	Tsp45I	Tsp509I	TspRI			
```